

What is claimed is:

1. A method of evaluating a solid phase for use in a dual bead assay, the method comprising the steps of:
  - 5 selecting a test solid phase;
  - binding a probe to the test solid phase in the presence or absence of a cross-linking agent;
  - determining a total amount of probe bound to the test solid phase in the presence or absence of a cross-linking agent;
  - 10 determining a percentage of probe bound covalently to the solid phase;
  - determining an amount of probe bound to the solid phase non-covalently; and
  - calculating a percentage of probe bound covalently to the solid phase, wherein if no less than a pre-determined minimum threshold of the probes is bound covalently, the solid phase is suitable for use in a dual bead assay.
- 15 2. The method according to claim 1 wherein said solid phase is a bead.
3. The method according to claim 2 wherein said bead is a magnetic bead.
- 20 4. The method according to claim 1 wherein said solid phase is a surface on a bio-disc.
5. The method according to claim 1 wherein said probe is nucleic acid.
- 25 6. The method according to claim 5 wherein said nucleic acid is double stranded.
7. The method according to claim 1 wherein said probe is a protein.
- 30 8. The method according to claim any of the claims 5, 6, or 7 wherein said probe further comprises a linker.
9. The method according to claim 8 wherein said linker is at least 1 polyethylene glycol moiety.

10. The method according to claim 8 wherein said linker is a polymer consisting of polyethylene glycol.

5           11. The method according to any of the claims 5, 6, 7, 9, or 10 wherein said probe is biotinylated.

12. The method according to claim 11 wherein said probe is quantified by an enzyme assay.

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13. The method according to claim 1 wherein said test solid phase is attached to a bio-disc.

14. The method according to claim 1 wherein the said minimum threshold for  
15 covalent probe binding is 50%.

15. The method according to claim 1 wherein the said minimum threshold for covalent probe binding is 80%.

20           16. A method for DNA conjugation onto a solid phase for determining the suitability of a test solid phase for use in a dual bead assay, said method comprising the steps of:

selecting a test solid phase;

conjugating a probe onto the test solid phase;

25           washing the solid phase employing a conjugate dilution buffer;

heat treating the solid to thereby remove the non-covalently bound probes,  
and;

calculating the percentage of probes bound covalently to the solid phase.

30           17. The method according to claim 16 wherein if no less than a predetermined minimum threshold of probe is bound covalently, the solid phase is suitable for use in a dual bead assay.

18. The method according to claim 17 wherein the said minimum threshold for covalent probe binding is 50%.

19. The method according to claim 17 wherein the said minimum threshold  
5 for covalent probe binding is 80%.

20. The method according to either claim 18 or 19 wherein said solid phase is a bead.

10 21. The method according to claim 20 wherein said bead is a magnetic bead.

22. The method according to claim 21 wherein said bead is a 3 $\mu$ m bead.

15 23. The method according to claim 22 wherein said conjugation is performed in the presence of a cross linking agent.

24. The method according to claim 23 wherein said cross-linking agent is EDC.

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25. The method according to claim 16 wherein said probe is single stranded.

26. The method according to claim 16 wherein said probe is double stranded.

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27. The method according to claim 16 wherein said conjugation is partially covalent.

28. The method according to claim 16 wherein said conjugation is  
30 completely covalent.

29. An optical bio-disc, comprising:  
a substrate having a tracking groove formed therein;

a reflective layer formed on at least a portion of said substrate so that an incident beam of electromagnetic energy may track along said groove;

an active layer associated with said substrate; and

a capture agent having an affinity for said active layer so that said capture agent is immobilized by said active layer so that a percentage of said capture agent bound covalently to said active layer may be calculated.

30. The optical bio-disc according to claim 29 wherein said capture agent is a strand of DNA.

31. The optical bio-disc according to claim 30 wherein said strand of DNA is a single strand of DNA.

32. The optical bio-disc according to claim 30 wherein said strand of DNA includes a double strand of DNA.

33. The optical bio-disc according to claim 29 wherein the capture agent is an antibody.

34. The optical bio-disc according to claim 29 wherein the capture agent is an antigen.

35. The optical bio-disc according to claim 29 wherein the capture agent is biotin.

36. The optical bio-disc according to claim 29 wherein the capture agent is streptavidin.

37. The optical bio-disc according to any one of claims 30, 31, 32, 33, 34, 35, or 36 wherein said active layer is formed from a polystyrene co-maleic anhydride.

38. The optical bio-disc according to claim 37 wherein said capture agent contains an active group that binds covalently to said active layer.

39. The optical bio-disc according to claim 38 wherein said active group is an amino group.

5           40. The optical bio-disc according to claim 38 wherein said capture agent binds to an anchor agent to thereby locate said anchor agent within said target zone.

10           41. The optical bio-disc according to claim 40 wherein said anchor agent is a DNA strand.

            42. The optical bio-disc according to claim 40 wherein said anchor agent is an antibody.

15           43. The optical bio-disc according to claim 40 wherein said anchor agent is an antigen.

            44. The optical bio-disc according to claim 40 wherein said anchor agent is biotin.

20           45. The optical bio-disc according to claim 40 wherein said anchor agent is streptavidin.

            46. The optical bio-disc according to claim 40 wherein said anchor agent is attached to a bead to thereby locate said bead within the target zone.

            47. The optical bio-disc according to claim 46 wherein said bead is a capture bead.

30           48. The optical bio-disc according to claim 46 wherein said bead is a reporter bead.

49. The optical bio-disc according to claim 47 wherein said capture bead and reporter bead are linked by a target molecule thereby forming a dual bead complex which is tethered to said capture agent within said target zone.

5 50. The optical bio-disc according to claim 49 wherein an incident beam of electromagnetic radiation inspects said dual bead complex.

51. An optical bio-disc, comprising:

a substrate having encoded information associated therewith, said encoded  
10 information being readable by a disc drive assembly to control rotation of the disc;

a target zone associated with said substrate, said target zone disposed at a predetermined location relative to said substrate;

an active layer associated with said target zone; and

a plurality of capture agents attached to said active layer so that when said  
15 substrate is rotated, said capture agents remain attached to said active layer to thereby maintain a number of said capture agents within said target zone so that when a dual bead complex including covalently bound probes is introduced into said target zone, said capture agent sequesters said dual bead complex therein to thereby allow detection of captured dual bead complex.

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52. The optical bio-disc according to claim 51 wherein said capture agent is a single stranded oligonucleotide sequence.

53. The optical bio-disc according to claim 51 wherein said capture agent is a  
25 double stranded oligonucleotide sequence.

54. The optical bio-disc according to claim 51 wherein said capture agent is an antibody.

30 55. The optical bio-disc according to claim 51 wherein said capture agent is an antigen.

56. The optical bio-disc according to claim 51 wherein said capture agent is biotin.

57. The optical bio-disc according to claim 51 wherein said capture agent is streptavidin.

5 58. The optical bio-disc according to any one of claims 52, 53, 54, 55, 56, or 57 wherein said capture agent contains an amino group.

59. The optical bio-disc according to claim 58 wherein said active layer is formed from a polystyrene-co-maleic anhydride.

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60. The optical bio-disc according to claim 59 wherein said amino group chemically reacts with said maleic anhydride to form a covalent bond thereby maintaining said capture agents within said target zone.

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61. The optical bio-disc according to claim 60 wherein said capture agent binds with an anchor agent to thereby locate said anchor agent within said target zone.

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62. The optical bio-disc according to claim 61 wherein the anchor agent is bound to one of two beads forming said dual bead complex which includes a capture bead and a reporter bead.

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63. The optical bio-disc according to claim 62 wherein said anchor agent is associated with said capture bead.

64. The optical bio-disc according to claim 62 wherein said anchor agent is associated with said reporter bead.

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65. The optical bio-disc according to claim 62 wherein said capture and reporter beads are linked together by a target agent to thereby form said dual bead complex.

66. The optical bio-disc according to claim 65 wherein said dual bead complex is immobilized within said target zone for inspection by an incident beam of electromagnetic radiation.

5           67. A method of preparing a dual bead assay for use in an optical bio-disc, said method comprising the steps of:

          providing a mixture of capture beads that have transport probes covalently bound thereto;

          suspending said mixture of capture beads in a hybridization solution;

10          adding to said mixture a target agent that hybridizes with said transport probes;

          adding to said mixture reporter beads including covalently bound signal probes attached thereto;

15          allowing said signal probes to hybridize with said target agent to thereby form a dual bead complex including at least one capture bead and one reporter bead;

          separating said dual bead complex from unbound reporter beads;

          removing from said mixture said unbound reporter beads; and

          loading said mixture including said dual bead complex into an optical bio-disc for analysis.

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68. The method according to claim 67 wherein said step of adding said target agent is performed before said step of adding said reporter beads.

25          69. The method according to either claim 67 or 68 wherein said target agent is a segment of genetic material.

70. The method according to claim 69 wherein said segment of genetic material is a single strand of DNA.

30          71. The method according to claim 69 wherein said segment of genetic material includes a portion of double stranded DNA.

72. The method according to claim 69 wherein said segment of genetic material is a single strand of RNA.



73. The method according to claim 69 wherein said segment of genetic material includes a portion of double stranded RNA.

5        74. The method according to claim 67 wherein said capture beads are magnetic and said separating step is performed by use of a magnet field.

75. The method according to claim 74 wherein said magnetic field is formed by a magnet.

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76. The method according to claim 74 wherein said magnetic field is formed by an electromagnet.

15        77. The method according to claim 67 including the further step of removing said hybridization solution for said mixture.

78. The method according to claim 77 including the further step of washing said dual bead complex to purify said mixture by further removing unbound material.

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79. The method according to claim 78 including the further step of adding a buffer solution to said mixture.

80. A method of preparing a dual bead assay for use in an optical bio-disc, said method comprising the steps of:

25        providing a mixture of capture beads having transport probes covalently attached thereto;

          suspending said mixture of capture beads in a hybridization solution;

          adding to said mixture a target agent that hybridizes with said transport probes;

30        allowing said transport probes to hybridize with said target agent to thereby form a hybridized partial complex including at least one capture bead;

          separating within said mixture said hybridized partial complex from unbound target agents;

adding to said mixture reporter beads including signal probes covalently attached thereto;

allowing said signal probes to hybridize with said target agent to thereby form a dual bead complex including at least one capture bead and one reporter bead;

5 separating said dual bead complex from unbound reporter beads;

removing from said mixture said unbound reporter beads; and

loading said mixture including said dual bead complex into an optical bio-disc for analysis.

10 81. The method according to claim 80 wherein said step of adding said target agent is performed before said step of adding said reporter beads.

82. The method according to either claim 80 or 81 wherein said target agent is a segment of genetic material.

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83. The method according to claim 82 wherein said segment of genetic material is a single strand of DNA.

20 84. The method according to claim 82 wherein said segment of genetic material includes a portion of double stranded DNA.

85. The method according to claim 82 wherein said segment of genetic material is a single strand of RNA.

25 86. The method according to claim 82 wherein said segment of genetic material includes a portion of double stranded RNA.

87. The method according to claim 80 wherein said capture beads are magnetic and said separating step is performed by use of a magnet field.

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88. The method according to claim 87 wherein said magnetic field is formed by a magnet.

89. The method according to claim 87 wherein said magnetic field is formed by an electromagnet.

90. The method according to claim 80 including the further step of removing  
5 said hybridization solution for said mixture.

91. The method according to claim 90 including the further step of washing said dual bead complex to purify said mixture by further removing unbound material.

10 92. The method according to claim 91 including the further step of adding a buffer solution to said mixture.

93. A method of testing for the presence of a target-DNA in a DNA sample by use of an optical bio-disc, said method comprising the steps of:

15 preparing a DNA sample to be tested for the presence of a target-DNA;  
preparing a plurality of reporter beads each having covalently attached thereto a plurality of strands of signal-DNA and an anchor agent, the target-DNA and the signal-DNA being complementary;

20 preparing a plurality of capture beads each having covalently attached thereto a plurality of transport-DNA, the target-DNA and transport-DNA being complimentary;

mixing said DNA sample, said plurality of reporter beads, and said plurality of capture beads to thereby form a test sample, the transport-DNA and the signal-DNA being non-complimentary;

25 allowing hybridization between said signal-DNA, any target-DNA, and transport-DNA existing in the DNA sample to thereby form a dual bead complex including at least one capture bead and one reporter bead;

removing from the test sample reporter beads and capture beads that are not associated with the dual bead complex;

30 depositing said test sample in a flow channel of an optical bio-disc which is in fluid communication with a target zone, the target zone including a plurality of capture agents each including an amino group that attaches to an active layer to immobilize the capture agents within the target zone;

allowing any anchor agent to bind with the capture agents so that reporter beads associated with the dual bead complex are maintained within the target zone; and

- 5 detecting any dual bead complexes in the target zone to thereby determine whether target-DNA is present in the DNA sample.

94. A method of testing for the presence of a target-DNA in a test sample by use of an optical bio-disc, said method comprising the steps of:

- preparing a test sample to be tested for the presence of a target-DNA;
- 10 preparing a plurality of reporter beads each having covalently attached thereto a plurality of strands of signal-DNA, the target-DNA and the signal-DNA being complementary;
- preparing a plurality of capture beads each having covalently attached thereto a plurality of transport-DNA and an anchor agent, the target-DNA and
- 15 transport-DNA being complimentary;
- depositing a plurality of capture beads and reporter beads in a mixing chamber, each of said reporter beads and said capture beads including signal-DNA and transport-DNA, respectively, being non-complimentary to each other;
- depositing said test sample in the mixing chamber of an optical bio-disc
- 20 which is linked to a target zone by a connecting flow channel allowing any target-DNA existing in the test sample to bind to the signal-DNA and the transport-DNA on the reporter and the capture bead, respectively, to thereby form a dual bead complex;
- rotating the optical bio-disc to cause the dual bead complex to move from the
- 25 mixing chamber through the flow channel and into the target zone, the target zone including a plurality of capture agents each including an amino group that attaches to an active layer to immobilize the capture agents within the target zone, said capture agent having affinity for the anchor agent;
- allowing any anchor agent to bind with the capture agent so that capture
- 30 beads associated with dual bead complex are maintained within the capture zone;
- removing from the target zone reporter beads that are free of any dual bead complex; and
- detecting any dual bead complex in the target zone to thereby determine whether target-DNA is present in the test sample.

95. A method of testing for the presence of a target-RNA in a test sample by use of an optical bio-disc, said method comprising the steps of:

preparing a test sample to be tested for the presence of a target-RNA;

5 preparing a plurality of reporter beads each having covalently attached thereto a plurality of strands of signal-DNA, the target-RNA and the signal-DNA being complementary;

10 preparing a plurality of capture beads each having covalently attached thereto a plurality of transport-DNA and an anchor agent, the target-RNA and transport-DNA being complimentary;

depositing a plurality of capture beads and reporter beads in a mixing chamber, each of said reporter beads and capture beads including the signal-DNA and the transport-DNA, respectively, being non-complimentary to each other;

15 depositing said test sample in the mixing chamber of an optical bio-disc which is linked to a target zone by a connecting flow channel allowing any target-RNA existing in the test sample to hybridize with the signal-DNA and the transport-DNA on the reporter and the capture bead, respectively, to thereby form a dual bead complex;

20 rotating the optical bio-disc to cause the dual bead complex to move from the mixing chamber through the flow channel and into the target zone, the target zone including a plurality of capture agents each including an amino group that attaches to an active layer to immobilize the capture agents within the target zone, said capture agent and said anchor agent having affinity to each other;

25 allowing any anchor agent to bind with the capture agent so that capture beads associated with dual bead complex are maintained within the capture zone;

removing from the target zone reporter beads that are free of any dual bead complex; and

detecting any dual bead complex in the target zone to thereby determine whether target-RNA is present in the test sample.

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96. A method of testing for the presence of a target-antigen in a test sample by use of an optical bio-disc, said method comprising the steps of:

preparing a test sample to be tested for the presence of a target-antigen;

preparing a plurality of reporter beads each having covalently attached thereto a plurality of signal-antibody, the signal-antibody having an affinity to epitopes on the target-antigen;

5 preparing a plurality of capture beads each having covalently attached thereto a plurality of transport-antibody and an anchor agent, the transport-antibody having affinity to epitopes on the target-antigen;

depositing the capture beads and the reporter beads in a mixing chamber, each of said reporter beads and capture beads including the signal-antibody and the transport-antibody, respectively, having no affinity to each other;

10 depositing said test sample in the mixing chamber of an optical bio-disc which is linked to a target zone by a connecting flow channel allowing any target-antigen existing in the test sample to bind to the signal-antibody and the transport-antibody on the reporter and the capture bead, respectively, to thereby form a dual bead complex;

15 rotating the optical bio-disc to cause the dual bead complex to move from the mixing chamber through the flow channel and into the target zone, the target zone including a plurality of capture agents each including an amino group that attaches to an active layer to immobilize the capture agents within the target zone;

allowing any anchor agent to bind with the capture agent so that capture  
20 beads associated with dual bead complex are maintained within the capture zone;

removing from the target zone reporter beads that are free of any dual bead complex; and

detecting any dual bead complex in the target zone to thereby determine whether target-antigen is present in the test sample.

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97. The method according to any one of claims 93, 94, 95, or 96 wherein the said dual bead complex is detected by directing a beam of electromagnetic energy from a disc drive assembly toward said target zone and analyzing electromagnetic energy returned from said target zones.

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98. A method of making an optical bio-disc for testing for the presence of a target-DNA in a DNA sample, said method comprising the steps of:

providing a substrate having a center and an outer edge;

encoding information on an information layer associated with the substrate, said encoded information being readable by a disc drive assembly to control rotation of the disc;

forming a target zone in association with said substrate, said target zone  
5 disposed at a predetermined location relative to said center of said substrate;

applying an active layer in said target zone;

depositing within said target zone, a plurality of strands of capture-DNA each including an amino group that covalently attaches to said active layer to immobilize said strands of capture-DNA within said target zone;

10 forming a flow channel in fluid communication with said target zone;

forming a mixing chamber in fluid communication with the flow channel;

depositing a plurality of reporter beads in the mixing chamber, each of said reporters including a signal-DNA that has an affinity for the target-DNA;

depositing a plurality of capture beads in the mixing chamber, each of said  
15 capture bead including a transport-DNA that hybridizes with a portion of the target-DNA and is complementary to said capture-DNA, the transport-DNA and signal-DNA being non-complimentary; and

designating an input site associated with the mixing chamber, the input site implemented to receive a DNA sample to be tested for the presence of any target-  
20 DNA, so that when the DNA sample is deposited in the mixing chamber hybridization occurs between the signal-DNA, the target-DNA, and the transport-DNA to thereby form a dual bead complex including at least one reporter bead and one capture bead, so that when the disc is rotated, the dual bead complex move into the target zone and hybridization occurs between the anchor-DNA and the  
25 capture-DNA to thereby place the dual bead complex in the target zone.

99. A method of making an optical bio-disc for determining the presence of a target-DNA in a test sample, said method comprising the steps of:

providing a substrate having a center and an outer edge;

30 encoding information on an information layer associated with the substrate, the encoded information being readable by a disc drive assembly to control rotation of the disc;

forming a target zone in association with the substrate, the target zone disposed at a predetermined location relative to the center of the substrate;

- applying an active layer in the target zone;  
 depositing within the target zone, a plurality of strands of capture-DNA each including an amino group that covalently attaches to the active layer to immobilize the strands of capture-DNA within the target zone; and  
 5 forming a flow channel in fluid communication with the target zone.

100. The method according to claim 99 wherein the flow channel is implemented to receive a test sample including sample-DNA, a plurality of reporter beads each having covalently attached thereto a plurality of strands of signal-DNA,  
 10 and a plurality of capture beads each having covalently attached thereto a plurality of strands of transport-DNA.

101. The method according to claim 100 wherein the capture-DNA and the signal-DNA are non-complementary, the transport-DNA and the capture-DNA are  
 15 complimentary.

102. The method according to claim 101 wherein the sample-DNA to be tested for the presence of a target-DNA is complementary to the transport-DNA and the signal-DNA so that when the test sample is deposited in the flow channel, a dual  
 20 bead complex including at least one reporter bead and one capture bead is formed, and when the disc is rotated the dual bead complex moves into the target zone and hybridization occurs between any transport-DNA and the capture-DNA thereby maintaining capture beads and dual bead complexes within the target zone.

- 25 103. A method of making an optical bio-disc for determining the presence of a target-antigen in a test sample, said method comprising the steps of:  
 providing a substrate having a center;  
 encoding information on an information layer associated with the substrate, the encoded information being readable by a disc drive assembly to control rotation  
 30 of the disc;  
 forming a target zone in association with the substrate, the target zone disposed at a predetermined location relative to the center of the substrate;  
 depositing an active layer in the target zone;



depositing in the target zone, a plurality of capture agents each including an amino group that covalently attaches to the active layer to immobilize the capture agents within the target zone; and

forming a flow channel in fluid communication with the target zone, the flow  
5 channel implemented to receive a test sample including target-antigen.

104. A method of using the disc made according to claim 103 wherein a plurality of reporter beads each having covalently attached thereto a plurality of signal-antibody, and a plurality of capture beads each having covalently attached  
10 thereto a plurality of transport-antibody are introduced into the flow channel.

105. The method according to claim 104 wherein the signal-antibody has no affinity for the capture agent, the transport-antibody has affinity for the capture agent, and the transport-antibody and the signal-antibody have affinity to different  
15 epitopes on the target-antigen so that when the test sample is deposited in the flow channel, a dual bead complex including at least one reporter bead and one capture bead is formed.

106. The method according to claim 105 wherein when the disc is rotated,  
20 the dual bead complex moves into the target zone and binding occurs between any transport-antibody and the capture agent to thereby maintain capture beads and dual bead complexes within the target zone.

107. A method of making an optical bio-disc to test for the presence of a  
25 target agent in a test sample, the method comprising the steps of:

providing a substrate having a center and an outer edge;

encoding information on an information layer associated with the substrate, the encoded information being readable by a disc drive assembly to control rotation of the disc;

30 forming a target zone in association with the substrate, the target zone disposed at a predetermined location relative to the center of the substrate;

depositing an active layer in the target zone;

depositing a plurality of capture agents in the target zone, each capture agent including an amino group that covalently attaches to the active layer to immobilize the capture agent within the target zone;

forming a flow channel in fluid communication with the target zone;

5 forming a mixing chamber in fluid communication with the flow channel;

depositing a plurality of reporter beads in the mixing chamber, each of the reporter beads having covalently attached thereto a plurality of signal probes, each of the signal probe having affinity to the target agent; and

10 depositing a plurality of capture beads in the mixing chamber, each of the capture beads having covalently attached thereto a plurality of transport probes and an anchor agent, each of the transport probe having affinity to the target agent, the transport probes and signal probes having no affinity toward each other, and the capture agents and the anchor agents having specific affinity to each other.

15 108. The method according to any one of claims 93, 94, 95, or 96 wherein the said dual bead complex is detected by directing a beam of electromagnetic energy from a disc drive assembly toward said target zone and analyzing electromagnetic energy returned from said target zones.

20 109. An optical bio-disc, comprising:

a substrate having a center and an outer edge, said substrate forming a distal layer of the bio-disc, said substrate having a top surface and a bottom surface relative to an interrogation beam of electromagnetic energy directed from a disc drive;

25 a reflective layer formed on the bottom surface of said substrate;

an active layer associated with said substrate and said reflective layer; and

a strand of capture DNA including an amino group which has an affinity for said active layer so that said amino group covalently attaches to said active layer to immobilize said strand of DNA in a target zone disposed between said center and

30 said outer edge.

110. The optical bio-disc according to claim 109 wherein said strand of capture DNA is complementary to a strand of anchor DNA which includes a dual

bead complex with at least a reporter bead and a capture bead that is detectable by said interrogation beam.

111. The optical bio-disc according to either claim 109 or 110 wherein said  
5 strand of capture DNA is a single strand of DNA.

112. The optical bio-disc according to either claim 109 or 110 wherein said  
strand of capture DNA includes a double strand of DNA.

10 113. The optical bio-disc according to either claim 109 or 110 wherein said  
active layer is formed from a modified polystyrene.

114. The optical bio-disc according to either claim 109 or 110 wherein said  
reflective layer is interposed between said substrate and said active layer.  
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115. The method according to any of the claims 98, 99, 103, or 107 wherein  
said removing step is performed by rotating the optical bio-disc.

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